REMARKS

Amendments

Claims 1 and 11 have been amended to specify that the nucleic acid encoding an interfering RNA (RNAi) molecule is inserted within the adenoviral VA1 coding sequence corresponding to a secondary stem loop structure. Support for this amendment can be found in, for example, Figure 2C.

Applicants submit that this amendment does not constitute new matter, and this entry is requested.

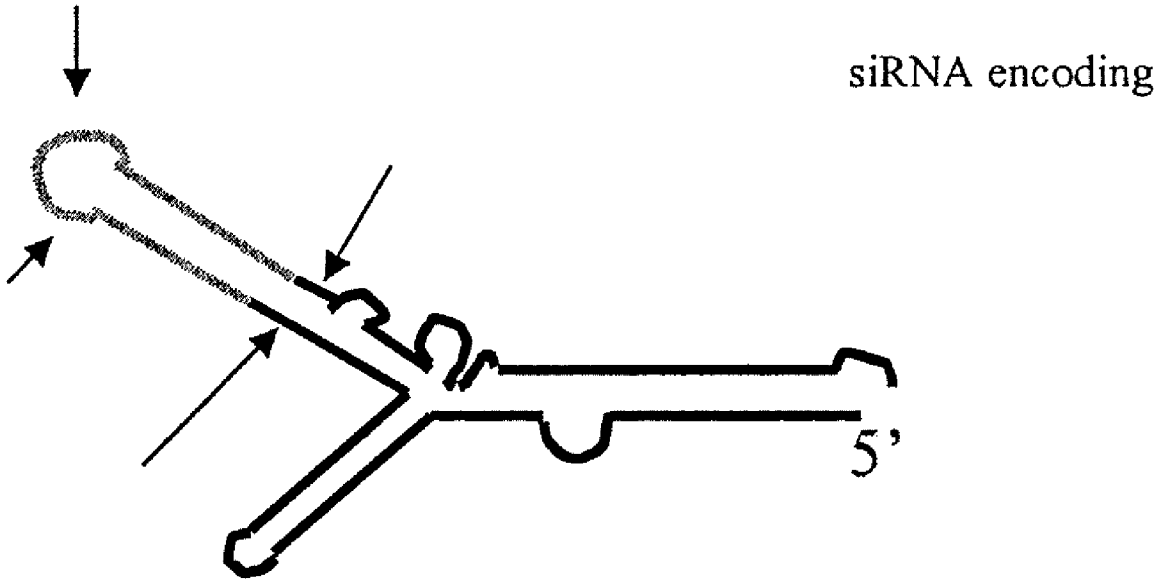
Summary of the Invention

In one aspect, the present invention is directed to an expression cassette which comprises an adenoviral VA1 gene and a nucleic acid encoding an interfering RNA (RNAi) molecule. The adenoviral VA1 gene comprises the adenoviral VA1 promoter and a coding sequence for the VA1 RNA. The nucleic acid is inserted within the adenoviral VA1 coding sequence corresponding to a secondary stem loop structure of the VA1 transcript as shown in Figure 2C. The nucleic acid encodes a hairpin siRNA (shRNA) or a precursor microRNA (precursor miRNA). The RNAi molecule is a substrate for Dicer (i) upon expression of the VA1 RNA which includes the RNAi molecule and (ii) after the RNAi molecule is processed out of the VA1 RNA. That is, in order to be a substrate for Dicer, the RNAi molecule must be removed from the VA1 RNA. As shown in Figure 3, the RNAi molecule is cleaved from the VA1 RNA (processed intermediate) and this processed intermediate is then a substrate for Dicer which produces the active siRNA or miRNA molecule. Hybridization of the active siRNA molecule to the mRNA in the cell leads to cleavage of the mRNA.

In a second aspect, the present invention is directed to a mammalian cell into which the above-defined expression cassette has been introduced.

As described above, Figure 2C illustrates the insertion of the claimed RNAi molecule into the secondary stem loop structure of the VA1 transcript. The following figure more clearly illustrates this insertion in the VA1 transcript in which the RNAi molecule is an shRNA. The darker

portion of the transcript represents the VA1 RNA and the lighter portion represents the shRNA. The longer arrows on the right illustrate the cleavage sites for processing the shRNA from the VA1 RNA and the shorter arrows on the left illustrate the cleavage sites for Dicer once the shRNA has been processed from the VA1 RNA as shown by the data in Figure 3.



Rejection Under 35 U.S.C. § 103(a)

The Examiner rejected claims 1, 2, and 11-16 under 35 U.S.C. 103(a) as being unpatentable over Agami et al. (US 7,241,618) taken with Doglio et al. (US 5,837,503) in further view of either Yu et al. (*Proc Natl Acad Sci USA* **99**:6047-6052, 2002) or Ambros (*Cell* **107**:823-826, 2001). The Examiner maintains that it would have been *prima facie* obvious to a person of ordinary skill in the art at the time of invention to combine these references to produce the claimed expression cassette and the claimed mammalian cell. Applicants traverse this rejection.

Independent claim 1, from which claims 2-3, 5-6, and 13-14 depend, is directed to an expression cassette comprising an adenoviral VA1 gene and a nucleic acid encoding an interfering RNA (RNAi) molecule. The adenoviral VA1 gene comprises the adenoviral VA1 promoter and a

coding sequence for the VA1 RNA. The nucleic acid is inserted within the adenoviral VA1 coding sequence corresponding to a secondary stem loop structure of the VA1 transcript. The nucleic acid encoding the RNAi molecule encodes a hairpin siRNA (shRNA) or a precursor microRNA (precursor miRNA). The claims further explicitly require that upon expression the VA1 RNA contains the RNAi molecule which is processed from the VA1 RNA to become a substrate for Dicer.

Independent claim 11, from which claims 12 and 15-16 depend, is directed to a mammalian cell into which the above expression cassette has been introduced.

The claimed RNAi molecule is a substrate for Dicer (i) upon expression of the VA1 RNA which includes the RNAi molecule and (ii) after the RNAi molecule is processed out of the VA1 RNA. Thus, in order to be a substrate for Dicer, the RNAi molecule must be removed from the VA1 RNA. It is clear from Figure 3 that the RNA molecule is cleaved from the VA1 RNA as a processed intermediate and this processed intermediate is then a substrate for Dicer which produces the active siRNA or miRNA molecule. Thus, Applicants submit that the specification teaches and the claims require that the RNAi molecule must be processed from the VA1 RNA before it can become a substrate for Dicer.

Applicants traverse the rejection on three grounds.

First, the references combined fail to teach each and every element of the claim. Each prior art cited by the Examiner fails to teach or suggest cleavage of an adenoviral VA1 transcript, which results in a processed intermediate that is a substrate for Dicer (as required by explicit claim language, "upon expression the VA1 RNA contains the RNAi molecule which is processed from the VA1 RNA to become a substrate for Dicer"). The Examiner has not cited any prior art nor pointed to any disclosure in the prior art which has been cited that teaches that a molecule inserted into a secondary stem loop structure of the VA1 transcript is cleaved, i.e., processed, from the VA1 transcript. Moreover, the cited references combined fail to teach or suggest inserting the nucleic acid encoding the RNAi molecule within the adenoviral VA1 coding sequence corresponding to a secondary stem loop structure of the VA transcript.

The Examiner continues to rely on the prior art for the same disclosures. The Examiner relies on Agami et al. as the primary reference for disclosure of (1) making and using an expression cassette comprising an adenoviral VA1 promoter operably linked to an siRNA molecule (i.e. shRNA) (col. 50-51; Figs. 8 and 10; claim 3), and(2) siRNA as a substrate for mammalian Dicer (col. 1-3). The Examiner concedes that "Agami et al. does not specifically teach using RNAi in the vector, wherein the RNAi is precursor miRNA," and "does not specifically teach the structural limitations of the claimed product." Office Action, p. 3.

Also, the Examiner relies on Doglio et al. for disclosure of an expression cassette comprising an oligonucleotide inserted between or outside the VA1 promoter (boxes A and B) or into the VA1 gene (col. 8, 10-15, and 19-22). The Examiner further relies on Yu et al. for disclosure of an RNA polymerase III vector comprising shRNA that can inhibit expression in mammalian cells. The Examiner relies on Ambros for disclosure of microRNA generally and that microRNAs likely participate in a wide range of genetic regulatory pathways.

The Examiner fails to point to any disclosure in Agami et al., Doglio et al., Yu et al., or Ambros that teaches cleavage of an adenoviral VA1 transcript containing an inserted molecule resulting in a processed intermediate that then is a substrate for Dicer. The references additionally fail to teach or suggest inserting the nucleic acid encoding the RNAi molecule within the adenoviral VA1 coding sequence corresponding to a secondary stem loop structure.

Second, there is no motivation to combine the references because prior art references explicitly teach away from the claimed invention. In other words, prior art references teach that the inhibitory RNA that had been inserted in a VA1 transcript is not cleaved from the VA1 transcript, but instead is active *within* the VA1 transcript, e.g. within the stem loop structure of the VA1 transcript. For such inhibitory RNA of the prior art, there need not be a processed intermediate that is a substrate for Dicer to produce the active siRNA or miRNA molecule.

For example, Cagnon et al. (*Antisense Nucl Acid Drug Dev* **10**:251-261, 2000) discloses a ribozyme that is introduced into the adenoviral VA1 gene at the top of the stem loop structure of the VA1 transcript and is active at this location. Rather than being cleaved from the VA1 transcript, the

ribozyme that has been inserted into the stem loop is protected from nuclease degradation. (See Figures 3A, 3B, 3D, 3E and 4A, p. 255.)

Similarly, Doglio et al. discloses an antisense oligonucleotide or ribozyme that is introduced into the adenoviral VA1 transcript. The antisense oligonucleotide or ribozyme is an "extrusion" relative to the VA1 transcript and is able to access mRNA. (See col. 3, lines 3-9; col. 4, lines 54-60; col. 5, lines 11-13; Figure 3 and 8 and col. 9 lines 38-40, 56-60 and col. 10, line 57- col. 11, line 8 and col. 12, lines 42-61). These molecules do not need to be processed from the VA1 RNA in order to be active.

In addition, Rossi et al. (U.S. Patent 6,100,087) discloses an inhibitory ribozyme that is introduced into the adenoviral VA1 gene at the top of the stem loop structure relative to the VA1 transcript and is active. The VA1 transcript is long-lived in the cytoplasm, i.e. not cleaved. (See col. 5, lines 20-22; Figure 9; col. 5, lines 22-33; Figures 6 and 7 and col. 1, lines 60-64 and col. 6, lines 20-41)

Each of these references teaches that the inhibitory RNA is not cleaved out of a VA1 transcript and that there need not be a processed intermediate that is a substrate for Dicer to produce the active siRNA or miRNA molecule. Thus, Applicants submit that these references teach away from the claimed invention.

Third, there is no motivation to combine the references because there is no reasonable expectation of success that the interfering RNA molecule that was inserted into the VA1 transcript would be cleaved out and become a substrate for Dicer.

Rossi et al. provides a clear illustration of this. See Figure 2A. Like the claimed invention, the inhibitory RNA is introduced into the adenoviral VA1 gene at the top of the stem loop structure relative to the VA1 transcript. Compare Figure 2A of Rossi et al to Figure 2C of the present application. *However*, the ribozyme coding sequence is not processed out of the VA1 transcript and yet is active. Thus, one skilled in the art would have had no reasonable expectation of success that a VA1 transcript containing an shRNA or miRNA molecule would be processed to produce a processed intermediate that is then a substrate for Dicer to produce the active siRNA or miRNA

molecule. Thus, the prior art teaches away from the claimed invention. See also Cagnon et al. (Figure 3B)

This lack of reasonable expectation of success is further supported by post-filing art. See Lu and Cullen (*J Virol* **78**:12868-12876, 2004), Andersson et al. (*J Virol* **79**:5556-5565, 2005), and Aparicio et al. (*J Virol* **80**:1376-1384, 2006). These post-filing art references disclose that it is only the 5' end and 3' end of the VA1 transcript that is cleaved by Dicer. The remainder of the VA1 transcript, i.e. the stem loop structure, is not cleaved by Dicer.

The Examiner cites Sharp (*Genes & Develop* **15**:485-490, 2001), Agami et al., and Brummelkamp et al. (*Science* **296**:550-553, 2002)) and Paddison et al. (*Genes & Develop* **16**:948-958, 2002) for disclosures of how siRNA is processed from dsRNA. Agami teaches a dsRNA that is cleaved by Dicer to form an active siRNA and the target transcript is cleaved upon hybridization of the active siRNA to the target transcript. See col. 1, line 63-col. 2, line 23. Sharp discloses a complex that generates the siRNA form short dsRNA recognizes the 3' termini of the duplex and internal cleavage occurs at a distance of approximately 22 nucleotides. See p. 385, left col to right col. According the Examiner, "one of ordinary skill in the art would reasonably expect the RNAi molecule would be cleaved out of the VA1 transcript and become a substrate for Dicer." Office Action, p. 6.

However, the references merely concern Dicer cleavage of linear dsRNA or essentially linear dsRNA, i.e., dsRNA with a short loop sequence. There is no teaching in any of this art to suggest that a dsRNA having the structure of the VA1 transcript would be cleaved by Dicer to release the shRNA or precursor miRNA molecule. In fact, Dicer does not cleave the VA1 transcript in a manner that would release the shRNA or miRNA contained in the secondary stem loop structure of the VA1 transcript.

The references only generally teach how siRNA is processed from dsRNA, i.e. that 1) Dicer cleaves the dsRNA, forming the active siRNA, and 2) the target mRNA, i.e. the target RNA transcript, is cleaved by the active siRNA molecule that hybridizes to the target mRNA. The Examiner does not explain any correlation (between how siRNA is generally processed from dsRNA

and how an RNAi molecule is cleaved out of a VA1 RNA transcribed from an expression cassette to become a substrate for Dicer) that would lead to any expectation of success.

There is no disclosure in Sharp, Agami et al., Brummelkamp et al., or Paddison et al. of the adenoviral VA1 transcript, cleavage of the VA1 transcript, and a resulting processed intermediate that is a substrate for Dicer to produce an active siRNA or miRNA molecule. Thus, these references are irrelevant to whether there is a reasonable expectation of success that an RNAi molecule is cleaved out of a VA1 RNA transcript to become a substrate for Dicer.

In summary:

1) Agami et al. taken with Doglio et al in further view of either Yu et al. or Ambros still fail to teach or suggest each and every element of independent claims 1 and 11, from which all other claims depend (i.e., a) inserting the nucleic acid encoding the RNAi molecule within the adenoviral VA1 coding sequence corresponding to a secondary stem loop structure of the VA1 transcript, and b) cleavage of an adenoviral VA1 transcript, which results in a processed intermediate that is a substrate for Dicer (as required by explicit claim language, "upon expression the VA1 RNA contains the RNAi molecule which is processed from the VA1 RNA to become a substrate for Dicer");

2) there is no motivation to combine the references because prior art references (Cagnon et al., Doglio et al., and Rossi et al.) explicitly teach away from the claimed invention by disclosure of inhibitory RNA is active *within* the VA1 transcript and teaching that there need not be a processed intermediate that is a substrate for Dicer to produce the active siRNA or miRNA molecule;

3) there is no motivation to combine the references because there is no reasonable expectation of success that the interfering RNA molecule inserted within the stem loop structure of the VA1 transcript would be cleaved out and become a substrate for Dicer, especially in view of Rossi et al and post-filing art.

In view of the above amendment and remarks, Applicants submit that the claimed subject matter is not rendered obvious by the combination of Agami et al., Doglio et al, and either Yu et al. or Ambros. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claim 1 and dependent claims 2 and 3 under 35 U.S.C. § 103(a) as being obvious over Agami et al. taken with Doglio et al. in further view of either Yu et al. or Ambros and in further view of Cagnon et al. (*Antisense Nucl Acid Drug Dev* **10**:251-261, 2000).

Dependent claim 2 adds the limitation to claim 1 that the RNAi molecule encoding nucleic acid is contained within a non-essential stem region of the coding sequence of the VA1 RNA. Dependent claim 3 further adds the limitation that the non-essential stem region contains a BstEII site.

According to the Examiner, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to combine the references to produce the claimed expression cassette wherein the nonessential stem region contains a BstEII site. In particular, "one of skill in the art would have been motivated to combine the teaching to clone the siRNA into the VA1 promoter of the expression cassette since the restriction site is found in an adenoviral VA1 promoter." Office Action, p. 7.

Applicants traverse this rejection for the same three grounds discussed above (supra p. 6-10).

Further, the new combination of references does not remedy all deficiencies or refute the arguments above. The Examiner cites Agami et al., Doglio, Yu et al. and Ambros as in the previous rejection. The Examiner cites Cagnon et al. for its disclosure of inserting an RNAi molecule (i.e., ribozyme) using a filled in NotI site that was ligated into the BstEII cleaved, filled in vector.

However, as discussed above (supra pp. 8-9), Cagnon et al. also discloses a ribozyme that is introduced into the adenoviral VA1 gene at the top of the stem loop structure and is active. Rather than being cleaved from the VA1 transcript, the ribozyme is protected from nuclease degradation as a result of insertion into the stem loop. Thus, the references together still fail teach or suggested a processed intermediate (as required by explicit claim language, "upon expression the VA1 RNA contains the RNAi molecule which is processed from the VA1 RNA to become a substrate for

Dicer."). Moreover, as discussed above (supra p. 7-8), Cagnon et al. teaches away from the claimed invention.

In view of the above amendment and remarks, Applicant submit that the claimed subject matter is not rendered obvious by the combination of Agami et al., Doglio et al, either Yu et al. or Ambros, and Cagnon et al. Withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1, 5 and 6 under 35 U.S.C. § 103(a) as being obvious over Agami et al. taken with Doglio et al. in further view of either Yu et al. or Ambros and in further view of Lorens (US 2004/0005593).

Dependent claim 5 adds the limitation to claim 1 that the RNAi molecule encoding nucleic acid comprises a loop containing from 4 to 9 bases. Dependent claim 6 further adds the limitation that the loop contains 8 bases.

The Examiner cites Agami et al., Doglio, Yu et al. and Ambros as in the previous rejection. The Examiner cites Lorens for its disclosure of an RNAi molecule having a loop containing at least 6 nucleotides. According to the Examiner, it would have been *prima facie* obvious to a person of ordinary skill in the art at the time of invention to combine the references to produce the claimed expression cassette. In particular, "one of skill in the art would have been motivated to combine the teaching to determine if there is an increase in the inhibition by using a common structure in a shRNA or precursor miRNA molecule to make the expression cassette." Office Action, p. 9.

Applicants traverse this rejection for the same three grounds discussed above (supra p. 6-10). Further, the new combination of references does not remedy all deficiencies or refute the arguments above.

In view of the above amendment and remarks, Applicant submit that the claimed subject matter is not rendered obvious by the combination of Agami et al., Doglio et al, either Yu et al. or Ambros, and Lorens. Withdrawal of this rejection is requested.

Conclusion

In view of the above amendments and remarks, Applicants believe that the present claims satisfy the provisions of the patent statutes and are patentable over the cited prior art. Reconsideration of the application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned to expedite the prosecution of the application.

Respectfully submitted,

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